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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 15/06, G01N 33/577, A61K 37/02, C12P 21/08, G01N 33/68

A2

(11) International Publication Number: **WO 92/08740**

(43) International Publication Date: 29 May 1992 (29.05.92)

(21) International Application Number: PCT/US91/08529

(22) International Filing Date: 14 November 1991 (14.11.91)

(30) Priority data: 613,326 14 November 1990 (14.11.90) US

(71) Applicant: THE ROCKEFELLER UNIVERSITY [US/US]; 1230 York Avenue, New York, NY 10021-6399 (US).

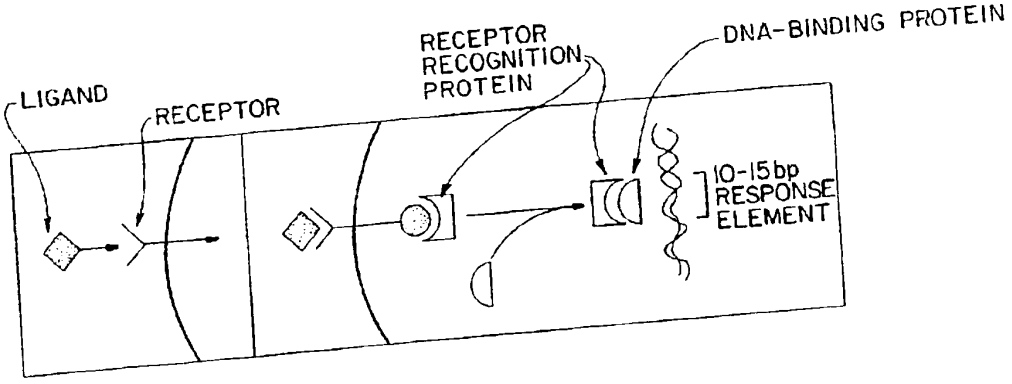
(72) Inventors: DARNELL, James, E., Jr. ; 95 Edgewood Avenue, Larchmont, NY 10538 (US). LEVY, David, E. ; 2 Washington Square Village, Apartment 13K, New York, NY 10012 (US).

(74) Agent: JACKSON, David, A.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: RECEPTOR RECOGNITION FACTOR AND METHODS OF USE THEREOF



(57) Abstract

A receptor recognition factor exists that recognizes the specific cell receptor to which a specific ligand has been bound, and that may thereby signal and/or initiate the binding of the transcription factor to the DNA site. The receptor recognition factor may itself be a part of a transcription factor or may interact with a transcription factor to cause it to activate and travel to the nucleus for DNA binding. The receptor recognition factor appears to be second-messenger-independent in its activity, as overt perturbations in second messenger concentrations are of no effect. The concept of the invention is illustrated by the results of studies conducted with interferon (IFN)-stimulated gene transcription, and particularly, the activation caused by IFNα. Additional observations derived from studies with IFNτ are also supportive.

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RECEPTOR RECOGNITION FACTOR AND METHODS OF USE THEREOF

RELATED PUBLICATIONS

The Applicants are authors or co-authors of several articles directed to the subject matter of the present invention. (1) [Applicants co-authored] "Interferon-Dependent Transcriptional Activation: Signal Transduction Without Second Messenger Involvement?" THE NEW BIOLOGIST 2(10):1-4, (1990); (2) [Applicants co-authored with X. Fu, D.S. Kessler and S.A. Veals] "ISGF3, The Transcriptional Activator Induced by Interferon α, Consists of Multiple Interacting Polypeptide Chains" PROC. NATL. ACAD. SCI., USA 87 (1990) (in press); and (3) [Applicant Levy co-authored with X. Fu, D.S. Kessler and S.A. Veals] "IFNα Regulates Nuclear Translocation and DNA-Binding Affinity of ISGF3, A Multimeric Transcriptional Activator" GENES AND DEVELOPMENT (in press). All of the above listed articles are incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a newly discovered factor and to methods and compositions including such factor or the antibodies or analogs thereof in assays and for diagnosing, preventing and/or treating cellular debilitation, derangement or dysfunction. More particularly, the present invention relates to a molecule or molecules that specifically control cellular activity through participation in gene activation, and to antibodies or to other entities specific thereto that may thereby selectively modulate such activity in mammalian cells.

BACKGROUND OF THE INVENTION

There are several possible pathways of signal transduction that might be followed after a polypeptide ligand binds to its cognate cell surface receptor. Within minutes of such ligand-receptor interaction, genes that were previously quiescent are rapidly transcribed (Murdoch et al., 1982; Larner et al., 1984; Friedman et al., 1984; Greenberg and Ziff, 1984; Greenberg et al., 1985). One of the most physiologically important, yet poorly understood, aspects of these immediate transcriptional responses is their specificity: the set of genes activated, for example, by platelet-derived growth factor (PDGF), does not completely overlap with the one activated by nerve growth factor (NGF) or tumor necrosis factor (TNF) (Cochran et al., 1983; Greenberg et al., 1985; Almendral et al., 1988; Lee et al., 1990). The interferons (IFN) activate sets of other genes entirely. Even IFNα and IFNγ, whose presence results in the slowing of cell growth and in an increased resistance to viruses (Tamm et al., 1987) do not activate exactly the same set of genes (Larner et al., 1984; Friedman et al., 1984; Celis et al., 1987, 1985; Larner et al., 1986).

The current hypotheses related to signal transduction pathways in the cytoplasm do not adequately explain the high degree of specificity observed in polypeptide-dependent transcriptional responses. The most commonly discussed pathways of signal transduction that might ultimately lead to the nucleus depend on properties of cell surface receptors containing tyrosine kinase domains [for example, PDGF, epidermal growth factor (EGF), colony-stimulating factor (CSF), insulin-like growth factor-1 (IGF-1); see Gill, 1990; Hunter, 1990) or of receptors that interact with G-proteins (Gilman, 1987). These two groups of receptors mediate changes in the intracellular concentrations of second messengers that,

in turn, activate one of a series of protein phosphokinases, resulting in a cascade of phosphorylations (or dephosphorylations) of cytoplasmic proteins.

It has been widely conjectured that the cascade of phosphorylations secondary to changes in intracellular second messenger levels is responsible for variations in the rates of transcription of particular genes (Bourne, 1988, 1990; Berridge, 1987; Gill, 1990; Hunter, 1990). However, there are at least two reasons to question the suggestion that global changes in second messengers participate in the chain of events leading to specific transcriptional responses dependent on specific receptor occupation by polypeptide ligands.

First, there is a limited number of second messengers (cAMP, diacyl glycerol, phospho-inositides, and Ca^{2+} are the most prominently discussed), whereas the number of known cell surface receptor-ligand pairs of only the tyrosine kinase and G protein varieties, for example, already greatly outnumbers the list of second messengers, and could easily stretch into the hundreds (Gill, 1990; Hunter, 1990). In addition, since many different receptors can coexist on one cell type at any instant, a cell can be called upon to respond simultaneously to two or more different ligands with an individually specific transcriptional response each involving a different set of target genes. Second, a number of receptors for polypeptide ligands are now known that have neither tyrosine kinase domains nor any structure suggesting interaction with G-proteins. These include the receptors for interleukin-2 (IL-2) (Leonard et al., 1985), IFNα (Uze et al., 1990), IFNγ (Aguet et al., 1988), NGF (Johnson et al., 1986), and growth hormone (Leung et al., 1987). The binding of each of these receptors to its specific ligand has been demonstrated to stimulate transcription of a specific set of genes.

For these reasons it seems unlikely that global intracellular fluctuations in a limited set of second messengers are integral to the pathway of specific, polypeptide ligand-dependent, immediate transcriptional responses.

A need therefore exists to further elucidate the mechanism for the control of transcriptional activity for animal cells, and to derive therefrom improved diagnostic techniques and therapeutic modalities that offer the opportunity for intervention to beneficially modulate cellular activity.

SUMMARY OF THE INVENTION

In accordance with the present invention, a receptor recognition factor has been postulated, that appears to interact directly with receptors that have been occupied by their ligand on cellular surfaces, and which in turn either activates or directly associates with transcription factors that enter the cells' nucleus and specifically binds on predetermined sites and thereby activates the genes. The receptor recognition factor appears to be proteinaceous in composition and is believed to be present in the cytoplasm. The recognition factor is not demonstrably affected by concentrations of second messengers, and likewise exhibits an apparent lack of interaction with tyrosine kinase domains, as well as structure that would enable an interaction with G-proteins. The recognition factor may comprise one or several proteinaceous substituents, the latter instance illustrated by the discussion of IFNα and IFNγ, later on herein.

The concept of the receptor recognition factor contemplates that specific factors exist for correspondingly specific ligands, such as tumor necrosis factor, nerve growth factor and the like, as described

earlier. Accordingly, the exact structure of each receptor recognition factor will understandably vary so as to achieve this ligand and activity specificity. It is this specificity and the direct involvement of the present receptor recognition factor in the chain of events leading to gene activation, that offers the promise of a broad spectrum of diagnostic and therapeutic utilities.

The present invention naturally contemplates several means for preparation of the recognition factor, including where applicable known genetic replicative techniques, and the invention is accordingly intended to cover such synthetic preparations within its scope.

The isolation of the cDNA amino acid sequence will facilitate the reproduction of the recognition factor by recombinant genetic techniques as discussed in detail hereinafter. Thus, the DNA sequence encoding the present recognition factor or analogs thereof can be used to construct vectors for expression in host systems by recombinant DNA techniques.

The invention includes an assay system for screening of potential drugs effective to modulate transcriptional activity of target mammalian cells by interrupting the recognition factor. In one instance, the test drug could be administered to a cellular sample or extract with the recognition factor, to determine its effect upon the binding activity of the recognition factor to either the sample or extract, or to the test drug, by comparison with a control.

The assay system could more importantly be adapted to identify drugs or other entities that are capable of binding to the receptor recognition and/or transcription factors or proteins, either in the cytoplasm or in the nucleus, thereby inhibiting transcriptional activity.

Such assay would be useful in the development of drugs that would be specific against particular cellular activity. For example, such drugs might be used to modulate cellular response to shock, or to treat other pathologies.

The present invention likewise extends to the development of antibodies against the receptor recognition factor, including naturally raised and recombinantly prepared antibodies. Such antibodies could include both polyclonal and monoclonal antibodies prepared by known genetic replicative techniques, as well as antibodies including other functionalities suiting them for additional diagnostic use conjunctive with their capability of modulating transcriptional activity.

The activity of the recognition factor or of the specific polypeptide believed to be causally connected thereto may be followed directly by the assay techniques discussed later on, through the use of an appropriately labeled quantity of the recognition factor or antibodies or analogs thereof. Alternately, the recognition factor can be used to raise binding partners or antibodies that could in turn, be labeled and introduced into a cellular mass containing a sample withdrawn from a mammalian host, to test for the presence of transcriptional activity, and to thereby assess the state of the host from which the medium was drawn.

Thus, both the receptor recognition factor and any antibodies that may be raised thereto, are capable of use in connection with various diagnostic techniques, including immunoassays, such as a radioimmunoassay, using for example, an antibody to the receptor recognition factor that has been labeled by either radioactive addition, reduction with sodium borohydride, or radioiodination.

In an immunoassay, a control quantity of the antibodies thereto, or the like may be prepared and labeled with an enzyme, a specific binding partner and/or a radioactive element, and may then be introduced into a cellular sample. After the labeled material or its binding partner(s) has had an opportunity to react with sites within the sample, the resulting mass may be examined by known techniques, which may vary with the nature of the label attached.

In the instance where a radioactive label, such as the isotopes ^{14}C, ^{131}I, ^{3}H, ^{125}I and ^{35}S are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric or gasometric techniques known in the art.

The present invention includes an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of the recognition factor. The system or test kit may comprise a labeled component prepared by one of the radioactive and/or enzymatic techniques discussed herein, coupling a label to the recognition factor, and one or more additional immunochemical reagents, at least one of which is a free or immobilized ligand, capable either of binding with the labeled component, its binding partner, one of the components to be determined or their binding partner(s).

In a further embodiment, the present invention relates to certain therapeutic methods which would be based upon the activity of the recognition factor, its subunits, or upon agents or other drugs determined to possess the same activity. A first therapeutic method is associated with the prevention of the manifestations of conditions causally related to or following from the binding

activity of the recognition factor or its subunits, and comprises administering an agent capable of modulating the production and/or activity of the recognition factor or subunits thereof, either individually or in mixture with each other in an amount effective to prevent the development of those conditions in the host.

More specifically, the therapeutic method generally referred to herein could include the method for the treatment of various pathologies or other cellular dysfunctions and derangements by the administration of pharmaceutical compositions that may comprise effective inhibitors or enhancers of activation of the recognition factor or its subunits, or other equally effective drugs developed for instance by a drug screening assay prepared and used in accordance with a further aspect of the present invention.

Accordingly, it is a principal object of the present invention to provide a receptor recognition factor its subunits in purified form that exhibits certain characteristics and activities associated with transcriptional promotion of cellular activity.

It is a further object of the present invention to provide antibodies to the receptor recognition factor and its subunits, and methods for their preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting the presence of the and its subunits in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

It is a further object of the present invention to provide a method and associated assay systen for screening substances such as drugs, agents and the like,

potentially effective in either mimicking the activity or combating the adverse effects of the recognition factor and/or its subunits in mammals.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the recognition factor or subunits thereof, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the recognition factor or its subunits, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon the recognition factor, its subunits, their binding partner(s), or upon agents or drugs that control the production, or that mimic or antagonize the activities of the recognition factors.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing description which proceeds with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a block diagram broadly depicting the concepts of the present invention.

FIGURE 2 is a block diagram specific to cellular interaction involving IFNα.

DETAILED DESCRIPTION

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

The terms "receptor recognition factor" and "recognition factor" may be used herein interchangeably, and as used throughout the present application and claims refer to proteinaceous material including single or multiple proteins having the profile of activities set forth herein and in the Claims. Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of the complex or its named subunits. Also, the terms "receptor recognition factor" and "recognition factor" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences.

A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses, inter alia, polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in

further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and $F(ab')_2$ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. (The disclosures of the art cited herein are hereby incorporated by reference.) Fab' antibody molecule portions are also well-known and are produced from $F(ab')_2$ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one

species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in the S phase activity of a target cellular mass, or other feature of pathology such as for example, elevated blood pressure, fever or white cell count as may attend its presence and activity.

In its primary aspect, the present invention concerns the identification of a receptor recognition factor that is believed to be present in cytoplasm and that serves as a signal transducer between a particular cellular receptor having bound thereto an equally specific polypeptide ligand, and the comparably specific transcription factor that enters the nucleus of the cell and interacts with a specific DNA binding site for the activation of the gene to promote the predetermined response to the particular polypeptide stimulus. Thus, it is postulated that specific and individual receptor recognition factors exist that correspond to known stimuli such as tumor

necrosis factor, nerve growth factor, platelet-derived growth factor and the like. Specific evidence of this is set forth with respect to interferon-α (IFNα).

The present receptor recognition factor is likewise noteworthy in that it appears to be not demonstrably affected by fluctuations in second messenger activity and concentration. The receptor recognition factor appears to lack perceptible interaction with tyrosine kinase domains and is likewise distinguishable in the perceived absence of interaction with G-proteins. This would eliminate the possibility that the present receptor recognition factor is a second messenger.

The possibilities both diagnostic and therapeutic that are raised by the existence of the receptor recognition factor derive from the fact that it appears to participate in direct and causal protein-protein interaction between the receptor that is occupied by its ligand, and those factors that thereafter directly interface with the gene and effect transcription and accordingly gene activation. As suggested earlier and elaborated further on herein, the present invention contemplates pharmaceutical intervention in the cascade of reactions in which the receptor recognition factor is implicated, to modulate the activity initiated by the stimulus bound to the cellular receptor.

Thus, in instances where it is desired to reduce or inhibit the gene activity resulting from a particular stimulus or factor, an appropriate inhibitor of the receptor recognition factor could be introduced to block the interaction of the receptor recognition factor with those factors causally connected with gene activation. Correspondingly, instances where insufficient gene activation is taking place could be remedied by the introduction of additional quantities of the receptor

recognition factor or its chemical or pharmaceutical cognates, analogs, fragments and the like.

As discussed earlier, the recognition factor or its binding partner(s) or other ligands or agents exhibiting either mimicry or antagonism to the recognition factor or control over its production, may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated specific transcriptional stimulation for the treatment thereof. A variety of administrative techniques may be utilized, among them parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of the recognition factor or its subunits may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of the recognition factor and/or its subunits may possess certain diagnostic applications and may for example, be utilized for the purpose of detecting and/or measuring conditions such as viral infection or the like. In particular, the recognition factor or its subunits may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with

Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against recognition factor peptides can be screened for various properties; i.e., isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of the recognition factor or its subunits. Such monoclonals can be readily identified in recognition factor activity assays. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant recognition factor is possible.

Preferably, the anti-recognition factor antibody used in the diagnostic methods of this invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-recognition factor antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions of whole antibody molecules.

As suggested earlier, the diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an anti-recognition factor antibody, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the anti-recognition factor antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions or whole antibody molecules. As previously discussed, patients capable of benefiting from this method include those suffering from cancer, a pre-cancerous lesion, a

viral infection or other like pathological derangement. Methods for isolating the recognition factor and inducing anti-recognition factor antibodies and for determining and optimizing the ability of anti-recognition factor antibodies to assist in the examination of the target cells are all well-known in the art.

Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or $F(ab')_2$ portions of useful antibody molecules, can be prepared using the hybridoma technology described in Antibodies - A Laboratory Manual, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated herein by reference. Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with a recognition factor-binding portion thereof, or recognition factor, or an origin-specific DNA-binding portion thereof.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact with the present recognition factor and their ability to inhibit specified transcriptional activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period

sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., Virol. 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal anti-recognition factor antibodies are also well-known in the art. See Niman et al., Proc. Natl. Acad. Sci. USA, 80:4949-4953 (1983). Typically, the present recognition factor or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen in the before described procedure for producing anti-recognition factor monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with the recognition factor peptide analog and the present recognition factor.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of a receptor recognition factor, polypeptide analog thereof or fragment thereof, as described herein as an active ingredient. In a preferred embodiment, the composition comprises an antigen capable of modulating the specific binding of the present recognition factor within a target cell.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage

for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of recognition factor binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

As used herein, "pg" means picogram, "ng" means nanogram, "ug" or "μg" mean microgram, "mg" means milligram, "ul" or "μl" mean microliter, "ml" means milliliter, "l" means liter.

It is further intended that receptor recognition factor analogs may be prepared from nucleotide sequences of the protein complex/subunit derived within the scope of the present invention. Analogs, such as fragments, may be produced, for example, by pepsin digestion of receptor

recognition factor material. Other analogs, such as muteins, can be produced by standard site-directed mutagenesis of receptor recognition factor coding sequences. Analogs exhibiting "receptor recognition factor activity" may be identified by known in vivo and/or in vitro assays.

As mentioned above, a DNA sequence encoding receptor recognition factor can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the receptor recognition factor amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, Nature, 292:756 (1981); Nambair et al., Science, 223:1299 (1984); Jay et al., J. Biol. Chem., 259:6311 (1984).

Synthetic DNA sequences allow convenient construction of genes which will express receptor recognition factor analogs or "muteins". Alternatively, DNA encoding muteins can be made by site-directed mutagenesis of native receptor recognition factor genes or cDNAs, and muteins can be made directly using conventional polypeptide synthesis.

A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael C. Griffith, Peter G. Schultz, Science, 244: 182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

The present invention also relates to a variety of diagnostic applications, including methods for detecting the presence of stimuli such as the earlier referenced

polypeptide ligands, by reference to their ability to elicit the activities which are mediated by the present receptor recognition factor. As mentioned earlier, the receptor recognition factor can be used to produce antibodies to itself by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the presence of particular transcriptional activity in suspect target cells.

As described in detail above, antibody(ies) to the receptor recognition factor can be produced and isolated by standard methods including the well known hybridoma techniques. For convenience, the antibody(ies) to the receptor recognition factor will be referred to herein as Ab_1 and antibody(ies) raised in another species as Ab_2.

The presence of receptor recognition factor in cells can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful utilize either the receptor recognition factor labeled with a detectable label, antibody Ab_1 labeled with a detectable label, or antibody Ab_2 labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the particle is labeled, and "RRF" stands for the receptor recognition factor:

A. $RRF^* + Ab_1 = RRF^*Ab_1$

B. $RRF + Ab^* = RRFAb_1^*$

C. $RRF + Ab_1 + Ab_2^* = RRFAb_1Ab_2^*$

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S. Patent

Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody", or "DASP" procedure.

In each instance, the receptor recognition factor forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.

It will be seen from the above, that a characteristic property of Ab_2 is that it will react with Ab_1. This is because Ab_1 raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab_2. For example, Ab_2 may be raised in goats using rabbit antibodies as antigens. Ab_2 therefore would be anti-rabbit antibody raised in goats. For purposes of this description and claims, Ab_1 will be referred to as a primary or anti-receptor recognition factor antibody, and Ab_2 will be referred to as a secondary or anti-Ab_1 antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine and auramine. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

The receptor recognition factor or its binding partner(s) can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The

preferred isotope may be selected from ^{14}C, ^{131}I, ^{3}H, ^{125}I and ^{35}S.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, ß-glucuronidase, ß-D-glucosidase, ß-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in accordance with the present invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

Accordingly, a purified quantity of the receptor recognition factor may be radiolabeled and combined, for example, with antibodies or other inhibitors thereto, after which binding studies would be carried out. Solutions would then be prepared that contain various quantities of labeled and unlabeled uncombined receptor recognition factor, and cell samples would then be inoculated and thereafter incubated. The resulting cell monolayers are then washed, solubilized and then counted

in a gamma counter for a length of time sufficient to yield a standard error of <5%. These data are then subjected to Scatchard analysis after which observations and conclusions regarding material activity can be drawn. While the foregoing is exemplary, it illustrates the manner in which a receptor assay may be performed and utilized, in the instance where the cellular binding ability of the assayed material may serve as a distinguishing characteristic.

In a further embodiment of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of predetermined transcriptional activity or predetermined transcriptional activity capability in suspected target cells. In accordance with the testing techniques discussed above, one class of such kits will contain at least the labeled receptor recognition factor or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., "competitive", "sandwich", "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Accordingly, a test kit may be prepared for the demonstration of the presence or capability of cells for predetermined transcriptional activity, comprising:

(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of the present receptor recognition factor or a specific binding partner thereto, to a detectable label;

(b) other reagents; and

(c) directions for use of said kit.

More specifically, the diagnostic test kit may comprise:

(a) a known amount of the receptor recognition factor as described above (or a binding partner)

generally bound to a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;

(b) if necessary, other reagents; and

(c) directions for use of said test kit.

In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive", "sandwich", "double antibody", etc.), and comprises:

(a) a labeled component which has been obtained by coupling the receptor recognition factor to a detectable label;

(b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:

(i) a ligand capable of binding with the labeled component (a);

(ii) a ligand capable of binding with a binding partner of the labeled component (a);

(iii) a ligand capable of binding with at least one of the component(s) to be determined; and

(iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and

(c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the receptor recognition factor and a specific binding partner thereto.

In accordance with the above, an assay system for screening potential drugs effective to modulate the activity of the receptor recognition factor may be prepared. The receptor recognition factor may be

introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in the transcriptional activity of the cells, due either to the addition of the prospective drug alone, or due to the effect of added quantities of the known receptor recognition factor.

As mentioned earlier, the observation and conclusion underlying the present invention were crystallized from a consideration of the results of certain investigations with particular stimuli. Particularly, the present disclosure is illustrated by the results of work on protein factors that govern transcriptional control of IFNα-stimulated genes, as well as more recent data on the regulation of transcription of genes stimulated by IFNγ. The following is a brief summary of the conclusions of the studies on the stimulation of transcription by IFN.

I. Immediate Activation of Transcription by IFNα; Importance of ISGF3

Activation of genes by IFNα occurs within minutes of exposure of cells to this factor (Larner et al., 1984, 1986) and is strictly dependent on the IFNα binding to its receptor, a 49-kD plasma membrane polypeptide (Uze et al., 1990). However, changes in intracellular second messenger concentrations secondary to the use of phorbol esters, calcium ionophores, or cyclic nucleotide analogs neither triggers nor blocks IFNα-dependent gene activation (Larner et al., 1984; Lew et al., 1989). No other polypeptide, even IFNγ, induces the set of interferon-stimulated genes (ISGs) specifically induced by IFNα. In addition, it has been found that IFNγ-dependent transcriptional stimulation of at least one gene in HeLa cells and in fibroblasts is also strictly dependent on receptor-ligand interaction and is not activated by induced changes in second messengers (Decker

et al., 1989; Lew et al., 1989). These highly specific receptor-ligand interactions, as well as the precise transcriptional response, require the intracellular recognition of receptor occupation and the communication to the nucleus to be equally specific.

The activation of ISGs by IFNα is carried out by transcriptional factor ISGF3, or interferon stimulated gene factor 3. This factor is activated promptly after IFNα treatment without protein synthesis, as is transcription itself (Larner et al., 1986; Levy et al., 1988; Levy et al., 1989). ISGF3 binds to the ISRE, the interferon-stimulated response element, in DNA of the response genes (Reich et al., 1987; Levy et al., 1988), and this binding is affected by all of an extensive set of mutations that also affects the transcriptional function of the ISRE (Kessler et al., 1988a). Partially purified ISGF3 containing no other DNA-binding components can stimulate ISRE-dependent in vitro transcription (Fu et al., 1990). IFN-dependent stimulation of ISGs occurs in a cycle, reaching a peak of 2 hours and declining promptly thereafter (Larner et al., 1986). ISGF3 follows the same cycle (Levy et al., 1988, 1989). Finally, the presence or absence or ISGF3 in a variety of IFN-sensitive and IFN-resistant cells correlates with the transcription of ISGs in these cells (Kessler et al., 1988b).

ISGF3 is composed of two subfractions, ISGF3α and ISGF3γ, that are found in the cytoplasm before IFN binds to its receptor (Levy et al., 1989). When cells are treated with IFNα, ISGF3 can be detected in the cytoplasm within a minute, that is, some 3 to 4 minutes before any ISGF3 is found in the nucleus (Levy et al., 1989). The cytoplasmic component ISGF3γ can be increased in HeLa cells by pretreatment with IFNγ, but IFNγ does not by itself activate transcription of ISGs nor raise the concentration of the complete factor, ISGF3 (Levy et al.,

1990). The cytoplasmic localization of the proteins that interact to constitute ISGF3 was proved by two kinds of experiments. When cytoplasm of IFNγ-treated cells that lack ISGF3 was mixed with cytoplasm of IFNα-treated cells, large amounts of ISGF3 were formed (Levy et al., 1989). (It was this experiment that indicated the existence of an ISGF3γ component and an ISGF3α component of ISGF3). In addition, Dale et al. (1989) showed that enucleated cells could respond to IFNα by forming a DNA-binding protein that is probably the same as ISGF3.

The ISGF3γ component is a 48-kD protein that specifically recognizes the ISRE (Kessler et al., 1990; Fu et al., 1990). Three other proteins, presumably constituting the ISGF3α component, were found in an ISF3-DNA complex (Fu et al., 1990). The roles of, or the relationships among these three proteins are not yet known, but it is clear that ISGF3 is a multimeric protein complex. Since the binding of IFNα to the cell surface converts ISGF3α from an inactive to an active status within a minute, at least one of the proteins constituting ISGF3α must be affected promptly, perhaps by a direct interaction with the IFNα receptor.

The details of how the ISGF3γ component and the three other proteins are activated by cytoplasmic events and then enter the nucleus to bind the ISRE and increase transcription are not yet known. Further studies of the individual proteins, for example, with antibodies, are required. Nevertheless, it is clear that, within 10 minutes of IFNα treatment, there is more ISGF3 in the nucleus than in the cytoplasm and that the complete factor has a much higher affinity for the ISRE than the 48-kD ISGF3γ component by itself (Kessler et al., 1990).

Thus, and with reference to FIGURE 1, a reasonable scheme that involves possibly as few as three specific protein-protein interactions and one specific protein-DNA

interaction can be proposed for the events involved in IFNα-dependent signal transduction from the cell surface to the nucleus. First, the IFNα receptor at the cell surface binds its specific protein ligand; second, the internal domain of the bound receptor contacts, and directly activates, one (or more) of the three proteins constituting the ISGF3α complex; third, the activated complex moves into the nucleus and, by joining with the site-specific DNA-binding component, ISGF3γ, forms ISGF3, the high-affinity, DNA-binding transcriptional activator.

II. Immediate Activation of GBP by IFNγ

Although experiments on transcriptional stimulation by IFNγ have not progressed as far as those concerned with the IFNα response, a gene has been identified in HeLa cells and fibroblasts that promptly undergoes transcription when the cells are treated with IFNγ (Decker et al., 1989; Lew et al., 1989). The IFNγ stimulation, like that of IFNα-dependent transcriptional activation, is not affected by second-messenger perturbations. The gene under study, termed GBP because it encodes a guanylate-binding protein (unrelated to plasma membrane associated G-proteins), is also induced by IFNα, but the response to IFNα is only about 20% of that obtained with IFNγ.

The regulatory region of the GBP gene has been extensively mapped: the regulatory sequences include an ISRE, as do all other IFNα-stimulated genes (D. J. Lew, T. Decker, I. Strehlow, J. E. Darnell Jr., submitted). However, a major IFNγ-responsive binding site, GAS (gamma activating site) is different from the ISRE and binds a different protein termed GAF (gamma activating factor; T. Decker, D. J. Lew, J. Mirkovitch, J. E. Darnell Jr., submitted). The protein composition of GAF is not known, however two important facts about GAF are known. (i) In two cell types, HeLa cells and diploid fibroblasts, the

presence of GAF correlates with the transcriptional profile of the GBP gene. (ii) GAF can be formed entirely from preexisting cytoplasmic proteins, because cytoplasts of enucleated fibroblasts (treated or untreated with cycloheximide) produce GAF within a few minutes of being exposed to IFNγ. This is a second example of a cytoplasmic protein or set of proteins that, after receiving a signal from an occupied cell surface receptor, promptly transmits the signal to the nucleus to activate one specific gene and probably others as well.

DISCUSSION

Can Protein-Protein Interactions Explain Transcriptional Activations by PDGF?

It might be argued that even though IFNα- and IFNγ dependent transcriptional responses pass through specific "waiting" cytoplasmic proteins and do not involve global fluctuations in second messengers, they are the exception in the world of ligand-activated transcriptional responses. As already mentioned, neither the IFNα nor the IFNγ receptor has tyrosine kinase domains or multiple membrane spanning domains characteristic of receptors that interact with G-proteins and ultimately increase second messenger concentrations. However, many receptors do have these properties, and in some instances the transcriptional stimulation resulting from polypeptide ligands binding to these receptors is effectively mimicked by agents that act through changing second messenger concentrations.

In one well-studied example, PDGF stimulation of the c-fos gene is mimicked by phorbol ester treatment of cells. Phorbol esters presumably act by direct stimulation of protein phosphokinase C (Nishizuka, 1988). Furthermore, the same DNA element is required for the response to PDGF and to phorbol ester (Fisch et al., 1988); Gilman, 1987),

and the same DNA binding protein, SRF (serum response factor), possibly in conjunction with other proteins, is thought to be the transcriptional activator (Norman et al., 1988; Norman and Treisman, 1988; Prywes and Roeder, 1986). However, a regulated change in DNA-binding proteins at the C-fos promoter in response to PDGF has yet to be described. Does this mean that the pathway for the stimulation of transcription by PDGF is dependent on a total cellular increase in a protein kinase C-initiated cascade of phosphorylations, as is probably the case after phorbol ester stimulation? It is now known that the PDGF receptor has a number of other proteins associated with it, including phospholipase C (Morrison et al., 1990), a phosphoinositide kinase (Coughlin et al., 1989), and possibly a serine threonine kinase, c-Raf. It thus seems possible that occupation of a PDGF receptor activates transcription through the sequential activity of several bound proteins. Phorbol esters may mimic this pathway by inducing generalized phosphorylations that normally would be carried out in a locally controlled manner after PDGF occupation of its receptor.

A Model for Polypeptide-Dependent Gene Activation

It is proposed herein that each cell receptor whose occupation ultimately stimulates transcription through a DNA-binding protein that recognizes a specific consensus element in the DNA should also have a specific cytoplasmic counterpart, termed herein a receptor-recognition factor. Thus, and as illustrated schematically in Figure 1, the pathway that would conserve specificity would include three (at least) highly specific reactions: (i) the ligand-receptor interaction; (ii) the recognition of the internal domain of the bound receptor by a waiting cytoplasmic protein capable of assisting in the assembly of an active transcription complex; and (iii) the formation and

transport of the active transcription factor and the recognition in the nucleus of the specific DNA site. In the case of IFNα- activated transcription, candidates for all these proteins have been identified. In the case of PDGF activation of c-fos, it is now known that a protein called p62 can convert SRF from a loose to a tight DNA-binding factor (Shaw et al., 1989; Shaw, 1990). This or some other protein participating in an active SRF complex may be activated through interaction with the occupied PDGF receptor and may then become associated with DNA-bound SRF in the nucleus to regulate transcriptional activation.

The ability of DNA-binding proteins to recognize specific oligonucleotides is by now unquestioned, as is the specificity of receptor-ligand interactions. It is suggested that the receptor recognition factor is required to preserve the high degree of specificity in ligand-mediated transcriptional responses. Clearly, the receptor complex and proteins that recognize it could include kinases, and the proposed receptor recognition protein could be a substrate for such enzymes. However, specificity would be maintained in the recognition of the bound receptor by the receptor recognition factor. After activation of the required protein in the cytoplasm, movement of the transcription complex to the nucleus could well be mediated by shuttle proteins common to most, if not all, ligand-triggered transcriptional responses.

Transcription factors that occur in an inactive cytoplasmic form while they await signaling are found in several other instances in mammalian cells. For example, glucocorticoid receptors (Pratt et al., 1988; Picard and Yammamoto, 1987) are thought to reside in the cytoplasm complexed in an inactive form until their specific steroid ligand enters the cell by solubilization in the plasma membrane. The presence of the correct steroid

ligand leads to a change in the structure of the inactive cytoplasmic factor and the release of an active factor, which then enters the nucleus and thereby stimulates transcription. Specificity in the transcriptional response is inherent in the hormone-receptor specificity (Evans, 1988).

NF- B, and the transcriptional activator, also occurs in the cytoplasm of most cells in combination with an inhibitor protein, I B (Bacuerle and Baltimore, 1988a, b; Bacuerle and Baltimore, 1990). A number of different unrelated stimuli, including phorbol ester treatment, lipopolysaccharides, and polypeptide ligands (e.g., tumor necrosis factor, TNF) cause the dissociation of I B and activation of NF- B. Binding sites for NF- B are found in a wide range of genes, such as the immunoglobulin genes in lymphocytes, and genes that can function in many cells types, for example, the α interferon gene.

Thus, NF- B could represent a more widely active member of the hypothetical family of proteins that await signaling in the cytoplasm, and that may account for polypeptide ligand-specific transcriptional stimulation. Although NF- B can be activated in vivo by hyperstimulation of protein kinase C and in vitro by phosphorylation, it is believed that the cell surface receptor-mediated activation of NF- B does not depend on global fluctuations in second messenger concentrations. Instead, It is believed that it is more likely that ligand-dependent activation of NF- B requires a protein-protein interaction to release the factor from its inhibitory protein IB. It is not contended that the release of NF-I B from I B is unrelated to phosphorylation, but rather that the phosphorylation will be delivered locally from the participants in the bound receptor complex. For example, a specific kinase might be bound to each receptor that activates NF- B which contacted a liganded receptor.

Gene Regulation from the Cell Surface in Yeasts and Bacteria

If mammalian transcription factors that are activated through cell surface receptors do, in fact, pre-exist at least in part in the cytoplasm and have one subunit that can recognize a bound receptor, this would be no surprise to workers studying transcriptional activation of gene expression in bacteria and yeast. For example, the only response to polypeptide ligands that has been characterized in Saccharomyces cerevisiae, that is, the growth arrest of haploid cells by the mating pheromones, appears to be regulated through protein-protein and protein-DNA contacts. Pheromones bind to cell surface receptors that are linked to G-proteins. Ligand binding stimulates guanine nucleotide exchange by the α-subunit, causing release of the ß- and γ-subunits of the G-protein. It appears that free ß-γ complex is the direct activator of the pheromone response pathway, ultimately through activation of a transcription factor of genes whose activity is required for pheromone arrest (Whiteway et al., 1990).

Bacteria can respond to extracellular signals through specific transcriptional induction. For example, the response to low osmolarity requires induction of the porin OmpF (Iso et al., 1989). This gene is under control of a DNA-binding protein (OmpR) and a membrane protein (EnvZ) that senses osmolarity in the environment. The internal domain of EnvZ is a protein kinase whose substrate is OmpR. In a second instance, ToxR, a regulatory protein required for toxin production by Vibrio cholera is an integral membrane protein with a carboxyl-terminal, sequence-specific DNA-binding domain. This membrane protein can directly activate transcription (Miller et al., 1988).

Concluding Remarks

Signaling pathways leading to specific transcriptional responses from cell surface ligands must retain the specificity inherent in ligand-receptor interactions. It seems reasonable to propose that this strict connection between receptor and gene may be carried out through specific protein-protein contacts rather than through global changes in common small molecules. The cytoplasmic proteins activated in the IFNα-specific and possibly the IFNγ-specific transcriptional responses may be early examples of this class of recognition factors in mammalian cells.

This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A receptor recognition factor implicated in the transcriptional stimulation of genes in target cells in response to the binding of a specific polypeptide ligand to its cellular receptor on said target cell, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) a perceived absence of interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

2. The receptor recognition factor of Claim 1 which is proteinaceous in composition.

3. The receptor recognition factor of Claim 1 which is cytoplasmic in origin.

4. The receptor recognition factor of Claim 1 which is derived from mammalian cells.

5. The receptor recognition factor of Claim 1 labeled with a detectable label.

6. The receptor recognition factor of Claim 5 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

7. An antibody to a receptor recognition factor, the factor to which said antibody is raised having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) a perceived absence of interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

8. The antibody of Claim 7 comprising a polyclonal antibody.

9. The antibody of Claim 7 comprising a monoclonal antibody.

10. An immortal cell line that produces a monoclonal antibody according to Claim 9.

11. The antibody of Claims 7 labeled with a detectable label.

12. The antibody of Claim 11 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

13. A method for measuring the presence of a receptor recognition factor, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; a perceived absence of tyrosine kinase domains; and a perceived absence of interaction with G-proteins, wherein said receptor recognition factor is measured by:

A. preparing at least one sample of said receptor recognition factor;

B. preparing at least one corresponding antibody or binding partner directed to said receptor recognition factor samples;

C. placing a detectible label on a material selected from the group consisting of said receptor recognition factor samples and said antibody or binding partners thereto;

D. immobilizing a material selected from the group consisting of the material from Step C that is not labeled, and a biological sample from a mammal in which the presence and/or activity of said receptor recognition factor is suspected, on a suitable substrate;

E. placing the labeled material from Step C in contact with said biological sample, and in contact with the immobilized material;

F. separating the material from Step C that is bound to said immobilized material from material from Step C not bound to said immobilized material; and

G. examining said bound material for the presence of said labeled material.

14. The method of Claim 13 comprising a method for measuring the presence and activity of a polypeptide ligand associated with a given invasive stimulus in mammals.

15. The method of Claim 14 wherein said invasive stimulus is an infection.

16. The method of Claim 14 wherein said invasive stimulus is selected from the group consisting of viral infection, protozoan infection, tumorous mammalian cells, and toxins.

17. The method of Claim 13 comprising a method for determining the presence of invasive or idiopathic stimuli in mammals.

18. A method for measuring the binding sites for a receptor recognition factor, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; a perceived absence of interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins, wherein the binding sites for said receptor recognition factor are measured by:

A. providing at least one sample of said receptor recognition factor;

B. placing a detectible label on said receptor recognition factor sample;

C. placing the labeled receptor recognition factor sample in contact with a biological sample from a mammal in which binding sites for said receptor recognition factor are suspected;

D. examining said biological sample in binding studies for the presence of said labeled receptor recognition factor.

19. A method of testing the ability of a drug or other entity to modulate the activity of a receptor recognition factor which comprises culturing a colony of test cells which has a receptor for the receptor recognition factor in a growth medium containing the receptor recognition factor, adding the drug under test and thereafter measuring the reactivity of said receptor recognition factor with the receptor on said colony of test cells, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) a perceived absence of interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

20. An assay system for screening drugs and other agents for ability to modulate the production of a receptor recognition factor, comprising an observable cellular test colony inoculated with a drug or agent, and yielding a resulting supernatant, said supernatant then to be examined for the presence of said receptor recognition factor, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) a perceived absence of interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

21. A test kit for the demonstration of a receptor recognition factor in a eukaryotic cellular sample, comprising:

A. a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of said receptor recognition factor or a specific binding partner thereto, to a detectable label, said receptor recognition factor

having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; a perceived absence of interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins;

B. other reagents; and

C. directions for use of said kit.

22. The test kit of Claim 21 wherein said labeled immunochemically reactive component is selected from the group consisting of polyclonal antibodies to the receptor recognition factor, monoclonal antibodies to the receptor recognition factor, fragments thereof, and mixtures thereof.

23. A method of preventing and/or treating cellular debilitations, derangements and/or dysfunctions in mammals, comprising administering to a mammal a therapeutically effective amount of a material selected from the group consisting of a receptor recognition factor, an agent capable of promoting the production and/or activity of said receptor recognition factor, an agent capable of mimicking the activity of said receptor recognition factor, an agent capable of inhibiting the production of said receptor recognition factor, and mixtures thereof, or a specific binding partner thereto, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) a perceived absence of interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

24. A pharmaceutical composition for the treatment of cellular debilitation, derangement and/or dysfunction in mammals, comprising:

A. a therapeutically effective amount of a material selected from the group consisting of a receptor recognition factor, an agent capable of promoting the production and/or activity of said receptor recognition factor, an agent capable of mimicking the activity of said receptor recognition factor, an agent capable of inhibiting the production of said receptor recognition factor, and mixtures thereof, or a specific binding partner thereto, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; a perceived absence of interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins; and

B. a pharmaceutically acceptable carrier.

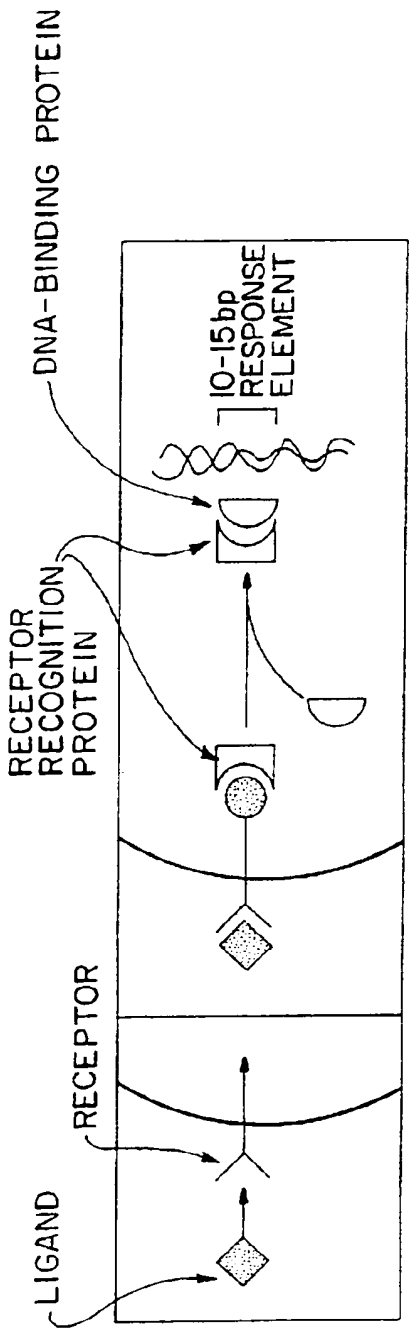
DNA-BINDING PROTEIN
10-15bp RESPONSE ELEMENT
RECEPTOR RECOGNITION PROTEIN
RECEPTOR
LIGAND

FIG. 1

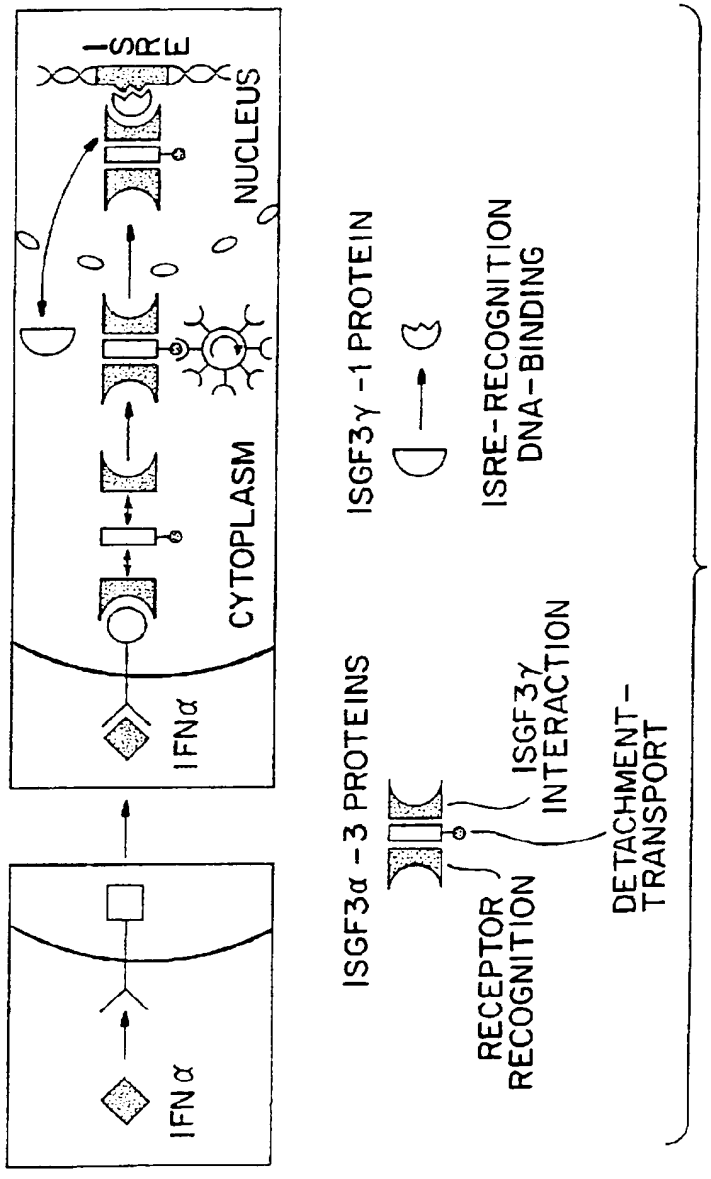
IFNα
IFNα
CYTOPLASM
NUCLEUS
ISRE
ISGF3α -3 PROTEINS
ISGF3γ -1 PROTEIN
RECEPTOR RECOGNITION
ISGF3γ INTERACTION
ISRE-RECOGNITION DNA-BINDING
DETACHMENT-TRANSPORT

FIG. 2